

**AN ELECTROPHORETIC STUDY OF
GENETIC DIFFERENTIATION IN
SOREX**

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Abstract Three karyotypic races of the common shrew <u>Sorex araneus</u> L. (Insectivora, Mammalia) have been chromosomally and electrophoretically investigated. The chromosomal differences are due to Robertsonian rearrangements and the races cannot be separated by any morphological criteria. A number of 225 animals from six populations in Sweden were analyzed for 27 loci by enzyme electrophoresis. Only one locus, <u>Mpi</u>, with three informative alleles, showed differences in gene frequencies between the races. The genetic distances (D) among the karyotypic races (0.004-0.019) are much smaller than the distances found among four different shrew species (0.32-2.23). Shrews are similar to other small mammals in the distribution of their heterozygosity (H) values (0.024-0.036). The three karyotypic races of <u>Sorex araneus</u> have a parapatric distribution with two zones of contact. One of these hybrid zones were electrophoretically investigated for three genetically variable loci (<u>Ada</u>, <u>EsB1</u> and <u>Mpi</u>) using 291 animals from populations far from, near and within the hybrid zone. The parallel frequency changes over the investigated region of the informative <u>Mpi</u> alleles indicated that gene flow exists between the karyotypic races. This gene flow must, however, be limited, since it has not been sufficient to equalize the gene frequency differences between the populations in the investigated region. The evolutionary processes that can take place in hybrid zones are discussed and in particular the possibility that the karyotypic races of <u>Sorex araneus</u> will in the future evolve into different species.			
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Date September 11, 1984

AN ELECTROPHORETIC STUDY OF
GENETIC DIFFERENTIATION
IN SOREX

INGRID FRYKMAN Sm

Akademisk avhandling som för avläggande av filosofie
doktorsexamen vid matematisk-naturvetenskapliga
fakulteten vid Universitet i Lund kommer att
offentligen försvaras å Genetiska Institutionens
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GENETIC DIFFERENTIATION
IN SOREX

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DEPARTMENT OF GENETICS
UNIVERSITY OF LUND SWEDEN

1984

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- III. Frykman, I. and Bengtsson, B.O. 1984. Genetic differentiation in Sorex. III. Electrophoretic analysis of a hybrid zone between two karyotypic races in Sorex araneus. — Hereditas 100: 259-270

References to these papers will be made by the above Roman numerals

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INTRODUCTION

How new species evolve is an intriguing question in population biology. Ever since Darwin (1859) presented his theory "On the Origin of Species by Means of Natural Selection", many different scenarios have been proposed for the precise details of how the process occurs (for general reviews see, for example, Mayr 1970, Bush 1975 and White 1978). Probably the most common mode of speciation, at least in sexually reproducing animals, is by geographic isolation, in the so called allopatric speciation model.

Allopatric speciation occurs if two populations, originally belonging to the same species, regain contact after a separation, during which they have become so genetically differentiated that there is a complete reproductive barrier between them. Given that they have also evolved such ecological differences that they can coexist, then they certainly have become two different species.

A very interesting evolutionary situation occurs if the populations come into contact before complete reproductive separation is obtained. If the hybrids have a reduced fitness and the vagility of the animals is limited, then the two populations will become parapatrically distributed with a more or less narrow hybrid zone. The two populations can after the formation of this hybrid zone evolve further in one of two contradictory ways:

1. A premating barrier may evolve between the animals belonging to the two populations, which leads to complete speciation. This barrier can be formed either as a result



of a continued accumulation of general genetic differences or due to a direct selection against the mating behaviour which leads to the formation of hybrids. The last suggestion was first made by Alfred Russel Wallace, see Wallace (1889). For a general discussion of the possibility for speciation between parapatrically distributed populations, see Futuyma and Mayr (1980).

2. The opposite evolutionary path such a situation may take, is that the gene flow through the hybrid zone will equalize the accumulated genetic differences between the populations with time. Their gene pools will then become mixed and the populations will again become genetically similar.

An example of this type of critical situation occurs when two populations have acquired different karyotypes during the time they have been separated from each other. Hybrids which are formed after contact between the populations has been regained, will then have a reduced fitness due to meiotic irregularities. Here is now a possibility for the evolutionary forces to act in the two opposite directions mentioned above. This situation is particularly interesting since a small number of chromosomal differences is sufficient to create a barrier to gene flow and thereby, perhaps, initiate the species formation. The idea that chromosome mutations can play an important role in the formation of new species underlies the suggestion of a special stasipatric mode of speciation (White 1968, 1978). The process demands, however, that such chromosomal barriers are stronger than normal physical barriers to gene flow, in order to be of primary importance for speciation; a claim which has been disputed by, among others, Barton (1979 a), Futuyma and Mayr (1980), Spirito et al. (1983) and Bengtsson (1984).

The present study

A number of hybrid zones between parapatric populations that differ chromosomally have been observed in nature (see the review of theoretical and empirical results on hybrid zones by Barton and Hewitt 1983), but only a few mammalian karyotypic hybrid zones have been investigated more closely. The common shrew is from this aspect an interesting species to study. Sorex araneus L. appears in Sweden in the form of three karyotypic races, with geographical distributions representing three parapatric populations with two zones of contact.

To study the genetic differentiation and the gene flow between the races, other markers than the chromosomal differences are needed since these are the genetic characters that define the races, and are likely to be subject to selection pressure arising from their effects on meiosis. Such genetic differences were looked for among the structural genes that can be analyzed by enzyme electrophoresis. The investigation of this genetic variation in the three karyotypic forms of Sorex araneus is presented in paper I.

To assess the amount of genetic differentiation found between the karyotypic races, a comparison with the differentiation that exists between different species of shrews was made. The electrophoretic comparison between Sorex araneus and three other sympatric shrew species is presented in paper II.

To assess the possibility of gene flow between the karyotypic races separate populations situated relatively far from, near and within a local contact zone between two of

the races were electrophoretically investigated. The results from this study are presented in paper III. A description of the specimens on which the reported work has been based is given in Table A1 in the Appendix.

The investigation of the genetic differentiation in Sorex in Sweden, of which a part is presented here, is a collaborative effort of a number of scientists:

Karl Fredga was the first to study the three karyotypic races of Sorex araneus in Sweden and to describe their distributions (Fredga 1973; Fredga and Nawrin 1977). In the present context he accounts for the chromosomal investigation of the populations in the contact region and of three other populations (see papers I and III). The detailed chromosomal information from the contact region will be presented by him in a later article (Fredga 1984).

Bengt Olle Bengtsson initiated the electrophoretic investigation and has been responsible for the choice of and the working out of the statistical methods that have been used. He also set out the mathematical admixture model in paper III.

Vibeke Simonsen has been responsible for developing the electrophoretic methods suitable for analyzing shrew material. The results described in papers I and II were obtained in her laboratory at the Department of Ecology and Genetics in Århus, while the electrophoretic runs for paper III were made in Lund.

My own responsibility has been to coordinate the practical aspects of the project, to organize the field work and the catching of shrews, to run the electrophoresis, and to collect and describe the obtained results in the articles and the present summary.

INVESTIGATED SHREW SPECIES

Taxonomy and distribution

All shrews belong to the family Soricidae (order Insectivora), which contain about 250 species (Corbet and Hill 1980). This study deals with four Swedish shrew species, three belonging to the genus Sorex and one to Neomys. The common shrew, Sorex araneus L., is almost everywhere in Sweden the most frequently occurring of the shrews. It can be found in the whole country except for the island of Gotland. The pygmy shrew, Sorex minutus L., and the European water shrew, Neomys fodiens Penn., are also distributed over most of the country but occur more sparsely. The fourth species, the masked shrew, Sorex caecutiens Laxm., occurs only in the northernmost parts of Sweden (Siivonen 1976). In local populations, S. araneus can appear sympatrically with the other shrew species mentioned, as we found when trapping them in nature (paper II).

The habitats occupied by shrews are quite varied, and comprise cultivated areas as well as moorlands, forests of different kinds and also the subalpine areas (Siivonen 1976).

In a study on the ecological interrelationships between S. araneus and S. minutus, Michielsen (1966) gathered a lot of valuable data on these species. The following description of the life of shrews is mainly based on her account and on the book by Crowcroft (1957). These authors have studied animals from Holland and Great Britain, res-

pectively, but many of their findings seem applicable to Swedish shrews.

Reproduction pattern and life history

A shrew is normally born in the spring or in the summer. The reproductive period of Swedish shrews stretches from March/April to August/September. Only very rarely can animals born in the spring mature the same season and produce offspring (Siivonen 1976). The overwintering young are active the whole winter and do not hibernate (Dowdswell 1959). The next spring the animals reach sexual maturity and the female produces her first litter after three weeks gestation (Crowcroft 1957). A female may produce up to three litters containing 3-10 young during this her only reproductive season. The first litter is large but the litter size declines somewhat (from mean value 6.7 to 5.1) as the breeding season proceeds (Michielsen 1966). The mean litter size found for S. araneus in northern Fennoscandia was 7.0 (Hansson et al. 1978). The parental generation dies out almost completely during the autumn and winter, resulting in a spring population mainly consisting of immature individuals. Consequently, each year there is a complete turnover of the population from one generation to the next, and generations are practically never reproductively mixed.

A species with this type of life cycle will show a great variation in population density over the year. In S. araneus there is an annual peak in August/September, but there is also a variation in population density be-

tween different years in the north of Sweden (Hörnfeldt 1982; Hansson et al. 1978). These fluctuations over years resemble the ones observed in other small mammals, as described by, for example, Andersson and Hansson (1974) and Nyholm and Meurling (1979).

Territories and dispersal pattern

Common shrews of both sexes are strictly territorial when sexually immature. Males and females live alone even in the breeding season. Only for a couple of hours during the mating period do the animals not defend their territories (Crowcroft 1957). Mean range sizes have been estimated by Michielsen to be $370-630 \text{ m}^2$.

The process of dispersal of the young in a litter can be divided into three phases:

1. The first spacing of the young takes place when the weaning period is over. The mother drives away her offspring just before she gives birth to her next litter. The young are by then almost fullgrown (Dehnel 1950) and they establish their first territories within a few weeks (Michielsen 1966). No family bond of any kind has been observed in S. araneus after the young have left their nest (Dehnel 1952). The young may disperse over considerable distances during this phase. It is difficult to measure these distances, but Michielsen found in her study that some animals dispersed at least 250-350 m. By successive trapping she once found a shrew which covered a distance of 800 m in a few weeks. This first spreading phase of the young is probably the most important one

for the dispersal of a litter.

2. The territory of a shrew is as a rule stationary, but a vacancy created nearby, by the disappearance of its previous inhabitant, will shortly afterwards be filled. Some additional dispersal can therefore take place during the winter.

3. After the winter, when the animals reach sexual maturity, the territorial borders become less strict. This is especially the case for males which then move over extensive areas to find mating partners. Also the activity of the females increases when the breeding season starts. They cover larger areas and sometimes even shift territory before giving birth to their first litter. Territory shifts in connection with new nest building for the second litter have also been observed (Michielsen 1966).

Addition of the distances covered during these three phases makes the vagility of a Sorex araneus individual between a few hundred meters and about one kilometer. Some individuals may move even further. Vagility is here defined as the mean distance between the point where an individual is born and the point at which it leaves its offspring (White 1978). These conclusions on the dispersal of a shrew litter is mainly based on the observations made by Michielsen on shrews from the Netherlands. The conditions are clearly different in the north of Sweden, in particular when the fluctuations over the years are taken into account, but one can expect that most of the facts given here about territories and dispersal pattern hold true also there.

KARYOTYPIC VARIABILITY IN SOREX ARANEUS

The common shrew is an unusual species in that it exhibits great variation in the number of chromosomes. Normally there is only one karyotype with a fixed chromosome number in a given species, a fact which can be seen from the checklist of chromosome numbers in eutherian (placental) mammals published by Matthey (1973 a, b). Out of more than 1300 species and subspecies listed, only for occasional ones a variation in the number of chromosomes has been reported.

Sex chromosomes

The araneus-arcticus taxonomic group, to which Sorex araneus belongs, (Hausser 1976) is characterized by the uncommon XX/XY_1Y_2 sex chromosome mechanism (Sharman 1956). Seven species have been recognized in this group (Fredga and Nawrin 1977). By this mechanism, males have always one chromosome more than the females. The mechanism has its origin in a translocation between the original X-chromosome and an autosome (Fig. 1). The homologous autosome is denoted Y_2 . The original Y-chromosome is denoted Y_1 and is smaller than Y_2 (Fredga 1970). The Y_2 is not directly involved in sex determination.

Chromosome polymorphism

Beside this dimorphism according to sexes, there is great variation in the number of chromosomes, as well as in their morphology, within the species. The chromo-

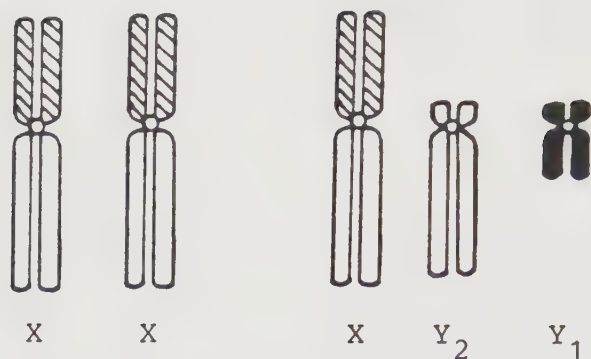


Fig. 1. Drawing of the sex chromosomes in Sorex araneus (female left, male right). The true X is shadowed, the true Y is black and autosomal parts are white. (After Fredga 1970).

some number may vary from 20 to 32 in the females and from 21 to 33 in the males (Fredga and Nawrin 1977) depending on whether the chromosome arms occur as metacentric (bi-armed) or telocentric (one-armed) chromosomes. This type of chromosomal variation is often called a Robertsonian polymorphism and has its origin in chromosomal centric fusion or fission events. Both of these events have probably occurred in the common shrew, but the order of the different events is not fully known (see Searle 1984 a).

In man, most centric fusions occur between the acrocentric chromosomes with nucleolar organizing regions (NORs). (For discussions on the formation of centric fusions and their relationship to chromosome associations see, for example, Hansson 1979 and Stahl et al. 1983). In Sorex araneus, the NOR regions are



Fig. 2. Chromosomes treated by silver staining. The NORs (arrowed) are located terminally in both homologues of two pairs. The animal representing the northern karyotype was collected in Ammarnäs. Female, $2n=26$. Scale bar $10\text{ }\mu\text{m}$.

not involved in the formation of metacentric chromosomes. Fig. 2 shows the chromosomes of a female S. araneus from the north of Sweden, where the NORs have been made visible with the silver staining technique according to Bloom and Goodpasture (1976). As seen from this figure and from the article by Olert and Schmid (1978), the NORs in S. araneus are situated at the terminal ends of the chromosomes and not close to the centromeric regions as they are in man. Thus, the fusion of chromosome arms into metacentric chromosomes has its basis in a different process in the shrews from that which occurs in humans.

Three karyotypic races

According to the combination of the chromosome arms, the species S. araneus can be divided into different karyotypic races (see the review by Searle 1984 a). In Sweden three such races have been described (Fredga 1973; Fredga and Nawrin 1977) which are called the southern (S), the central/middle (M) and the northern (N) karyotypic races according to their distribution over the country. In the southern part of Sweden (except for the island of Öland; Fredga pers. comm.) all investigated animals have had similar karyotypes with $2n=20(21)$. Here all chromosome arms are fused into metacentrics (see Fig. 1 in paper I). In the M-race which occupies the central part of Sweden, the number of chromosomes may vary since some chromosome arms sometimes occur as metacentric and sometimes as telocentric chromosomes. However, the basic difference between S- and M-race animals is that different chromosome arms are combi-

ned into different metacentrics in the two karyotypic races (see Fig. 3).

In the N-race different chromosome arms are again combined, as compared with the combinations in the southern and the central races (Fig. 3). In this race there is also a polymorphism for the number of telocentric chromosomes, as there is in the central race. The number of telocentric chromosomes is low in the southern part of the M-race distribution area and higher in the northern regions. In the N-race, the number of telocentrics may show the opposite pattern with more telocentrics in the southern than in the northern distribution area (Fredga 1982), but the evidence is here less clear. For a more detailed overview of the complicated chromosomal polymorphisms within and between the karyotypic races, see the introduction to paper I. Thus, in Sweden the species S. araneus is subdivided into three karyotypic races. These exist parapatrically with only

Race S	hi	jl	gm	ko	nq	pr
Race M	hi	jl	gm	kp	nr	oq
Race N	hn	jl	gm	ip	kq	or

Fig. 3. Possible chromosome arm combinations in the southern, central/middle and northern karyotypic races of Sorex araneus (Fredga and Nawrin 1977). Within the darker printed box the arm combinations are identical.

two zones of contact, since the southern and the northern races do not meet. A map showing the distribution of the three karyotypic races in Fennoscandia can be seen in Fredga and Nawrin (1977) and in Fredga (1984).

The differences in the karyotypes of the three races are not accompanied by any obvious morphological differences. A detailed investigation by Sulkava et al. (1983) on the upper tooth-row showed a slight clinal variation in relation to latitude. The unicuspid row increases in size while the molar row decreases going from the south to the north, giving a slightly longer snout to the shrews in the north. The variation found was, however, not directly connected with the karyotypic races.

Origin of the karyotypic races in Sweden

The common shrew has almost certainly colonized Sweden from at least two directions; the S-race from the south, and the N-race from the north-east. This post-glacial colonizing pattern was predicted by Ekman already in 1922. Support to the hypothesis has come from the facts that the chromosome number of Danish shrews from Zealand is the same as in the shrews from the south of Sweden (Meylan 1965), and that S. araneus from the north-eastern part of Finland shows the same karyotypic pattern as that found in animals from the northern part of Sweden (Halkka et al. 1974).

The origin of the central race is a more intriguing question. As far as I can see there are five logical possibilities for the evolution of the M-race:

1. The first possibility is that it spread to the central part of Sweden from the west after the last glaciation. The origin should then have been a population on the Norwegian coast that survived the last glaciation on ice-free refugies (Hanson 1956; Fredga and Nawrin 1977).

2. The second possibility is also based on immigration from the west. The difference here is that a small population entered the Norwegian south-west coast after the last glaciation from Jutland in Denmark (see Fig. 4). Sometime during its establishment this isolated population acquired the chromosome rearrangements we can see today in M-race animals, before it then spread eastwards following the rim of the melting ice.

3. A third possibility is that the central race has its origin in the southern race in Sweden. The chromosomal change should then have occurred in a small marginal population, isolated from the mother population until the chromosome mutations were fixed (homozygous). The new M-race has then spread northwards in Sweden.

4. It may be that the races were well differentiated before the deglaciation period, and that the M-race may have been the first race to colonize the country from the south (possibility 4a) or the north-east (possibility 4b). The S-race, or alternatively the N-race, should then have reached the country in a second wave of colonization.

5. The origin of the central race can, of course, also be the northern race with the same reasoning as under 3.

If the chromosomal differences that now exist between

the three races are studied (see Fig. 3) it can be seen that the S- and M-races are more similar to each other than the M- and N-races. The two first mentioned races differ in the arrangements of six chromosome arms, while

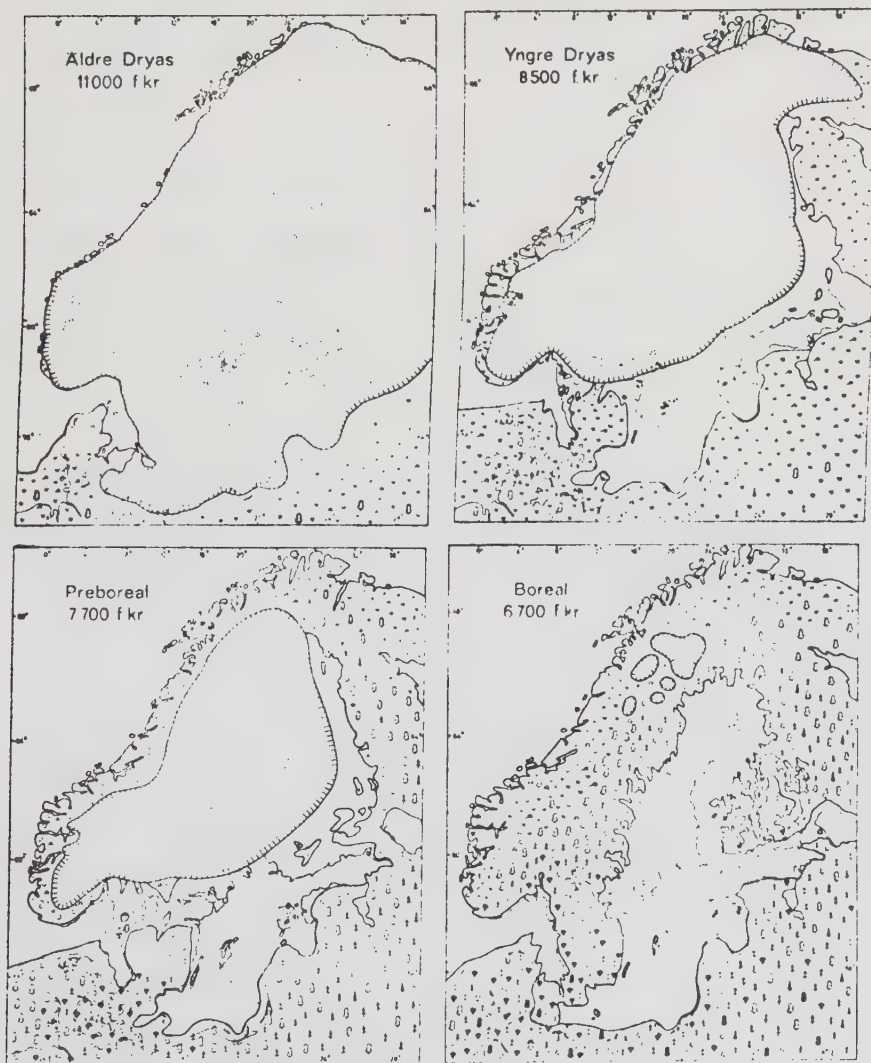


Fig. 4. Maps showing the deglaciation of Fennoscandia (from Berglund 1968).

the latter ones differ in eight chromosome arms. This fact favours the third possibility as the explanation of the origin of the M-race. It also favours the second possibility, if we assume that there originally existed an S-race population in Jutland. Also from the differences found at the Mpi enzyme locus (see Fig. 6 in paper I), it seems as if the S- and M-races are more similar to each other than the M- and N-races.

Given the available information I will here assume that the M-race has reached its present geographical distribution through one of the scenarios 1 to 4a. This then implies that the zone of contact between the M- and N-races represents a contact between animals that have been isolated from each other for an extensive stretch of time and/or distance. From the knowledge available about the disappearance of the inland ice from Sweden (Magnusson et al. 1963), see Fig. 4, one can estimate that the present type of contact between the races must have lasted for less than 10 000 years.

Meiosis and fertility in hybrids

The chromosomal differences between the races are such that hybrids will presumably have reduced fertility due to the complications that will occur in their meiosis. If, for example, an S-race animal with arm combinations ko, nq and pr, see Fig. 3, mates with an M-race animal with arm combinations kp, nr and oq, the meiosis in the hybrid will be quite complicated. After the chromosome arms have paired in pachytene, the result will be the formation of a

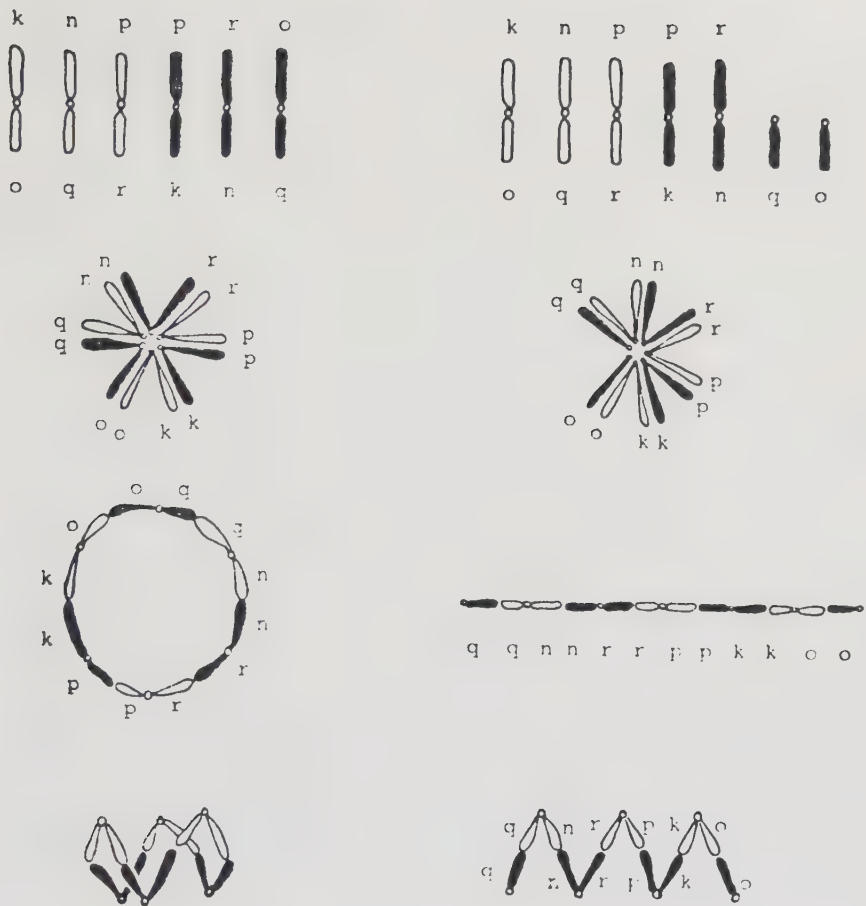


Fig. 5. Schematic diagram showing the formation of a ring of six or a chain of seven chromosomes in the meiosis of hybrids between the S- and the M-races. Chromosomes from the M-race are black and from the S-race are white.

ring consisting of six chromosomes (see Fig. 5). If one of the chromosomes (oq) occurs as two telocentric chromosomes in the hybrid, the arrangement in prophase will be a chromosome chain instead of a ring (see Fig. 5). These multivalents will produce balanced gametes only if a proper zig-zag configuration is obtained during the anaphase stage.

That such balanced gametes can be produced in a high frequency from complex chromosomal heterozygotes in shrews have been shown by Searle (1984 b).

Meiosis in a hybrid between an M- and an N-race animal may contain a ring of eight ~~or~~ a chain of nine chromosomes since these races differ at most by four metacentric chromosome pairs (see Fig. 3). However, the complications in the meiosis of these hybrids are probably somewhat less since the number of telocentric chromosomes are fairly high in the contact zone between the M- and N-races as mentioned above. One should also remember that not only cytological complications during the meiosis but also other genetic factors can affect the fitness of hybrids. Cattanaach and Mosely (1973) have, for example, showed that in hybrids between the two species Mus musculus and Mus posciavinus the greater part of the infertility observed results from genetic or minor chromosomal differences other than the Robertsonian differences by which the species differ.

We do not have any direct evidence concerning the fitness of the hybrids between the karyotypic shrew races in Sweden. However, given the large number of chromosomal changes involved in the racial differences and the clear parapatric distribution of the races (the narrow hybrid zones), it is reasonable to assume that the karyotypic hybrids carry a clear fitness disadvantage. To investigate whether any genes can pass through the karyotypic borders, the following electrophoretic investigation was undertaken.

ELECTROPHORETIC ANALYSIS OF THE KARYOTYPIC RACES

Results

The first thing to be investigated was the degree of genetic differentiation that exists between the three karyotypic races in Sorex araneus. For this reason a starch gel electrophoretic method was used. The genetic variation for 19 enzymes coded for by 27 loci was studied in six populations (two from each race). This investigation is presented in paper I. The six localities investigated were meant to represent "pure" karyotypic populations. The three localities Stensoffa (S-race), Uppsala (M-race) and Umeå (N-race) were chosen on the basis of earlier chromosomal investigations made by Fredga (1973) and Fredga and Nawrin (1977), while the three remaining populations from Stensjön (S-race), Mora (M-race) and Ammarnäs (N-race) were checked chromosomally and found to belong to the race they were expected to represent (see paper I). A total number of 225 animals were analyzed for the 27 loci in this investigation. Our intention was to search for diagnostic loci — loci where different alleles are fixed in different races — in order to separate the three karyotypic races on another genetic characteristic than the chromosomes.

The result of this analysis was that no diagnostic loci were observed. At 24 of the loci, no or only minor genetic variation was found (see Table 2 in paper I). All the rare alleles observed were present in heterozygous form. Three loci were found to be more variable, namely Ada, EsB1 and Mpi. The allele frequencies at these loci are given in Tables 3, 4 and 5 in paper I, where also a

discussion of the results obtained for Ada and EsB1 can be found.

The only locus with a substantial genetic difference between the races was Mpi. At this locus four alleles were observed, denoted A, B, C and D. The D allele was very rare and was found in only three heterozygous animals. The other three alleles exhibited clear frequency differences between the three races. Allele A was the most common one in the northern race while B was the most common allele in the central and the southern races. The C allele was not observed in the northern race at all (90 investigated animals), while it was quite common in the central and southern races. The allele frequencies in the different populations are given in Table 5 in paper I. In Fig. 6 in the same paper, the relative frequencies of alleles A, B and C are plotted in a trinomial diagram to make the frequency differences more visible. Here it is obvious that the populations from the S- and M-races are similar, while the populations from the N-race have clearly different allele frequencies.

For some enzymes (MDH, LDH, PGM and ES) attempts were made to detect more electrophoretic variation with an electrofocusing technique. Polyacrylamide gels covering different pH-ranges were used in order to separate the electrophoretic bands further. This analysis did not, however, lead to the detection of any new genetic variation.

In addition to the six populations that were analyzed for 27 loci, ten animals from Ammarnäs (N-race) and five animals from Nordingrå (M-race) were analyzed for alto-

gether 35 loci. The results from this investigation were used and mentioned in paper II, but a direct description of them has not been given before. Nordingrå is situated about 10 km from the east coast at the same latitude as Bispfors; see Fig. 6. The Nordingrå sample was chromosomally analyzed by Fredga and was found to belong to the central race (Fredga pers. comm.). The 35 loci investigated in the M- and N-races are:

1. The 27 loci reported in paper I;
2. A second locus for the ACO, IDH and ME enzymes that were not genetically interpreted in the previous investigation;
3. Five additional enzymes (CAT, FH, GPT, NP and SOD), each representing one locus (see Table 1 in paper II).

None of the eight newly analyzed loci showed any differences between the northern and the central karyotypic races. Thus, the change in allele frequencies at the Mpi locus between the northern and central races is the only racial difference found in our electrophoretic investigation.

Hardy-Weinberg proportions

The allele frequencies observed at the Mpi locus can be used to test whether the genotypes occur at Hardy-Weinberg proportions in the local populations. For this reason, the number of observed heterozygotes has been compared with the number of expected heterozygotes under

Hardy-Weinberg conditions. The method used is described in paper III. As is obvious from Table 5 in paper III, there is no significant deviation from the expected number of heterozygotes in any of the local populations. If anything, rather too many than too few heterozygotes have been observed. This effect is, however, not statistically significant.

There seems thus to be random mating in the investigated shrew populations, and we can exclude the possibility that the shrews move such short distances that there is a high frequency of consanguineous matings. Neither has any tendency to a Wahlund effect been found, i.e. that a sampled, presumed panmictic, population turns out to contain two or more subpopulations with different allele frequencies, causing what looks like a deficiency of heterozygotes (Wahlund 1928). Therefore, I will assume in later discussions that the shrew is an animal which practices random mating in local populations.

ANALYSIS OF A HYBRID ZONE

It is thus obvious that the three karyotypic races of Sorex araneus are very similar when judged on the basis of their electrophoretically detectable enzyme producing genes. There is, however, a locus, Mpi, for which the allele frequencies observed were different in the three races. The most striking difference was that the C allele never was observed in the northern race when 90 animals were investigated, while it was quite common both in the central and southern races. Also the most common allele was different in the northern race contra the other two. These differences at the Mpi locus between the northern and the central races made it possible to study if there is, or recently has been, gene flow across this karyotypic border.

During two summers animals were trapped at different localities from Bispfors (M-race) to Umeå (N-race), see Fig. 6. These two populations had previously been chromosomally analyzed by Fredga and been found to contain "pure" race animals only. By continuous chromosome analysis, made in the field by Fredga, eight populations situated far from, near and within the hybrid zone between the two karyotypic races were found and sampled for an electrophoretic investigation. A total number of 291 animals were collected from these populations and were electrophoretically analyzed for the three polymorphic loci Ada, EsB1 and Mpi. Joint karyotypic and electrophoretic information exists for 110 animals (Fredga 1984). The eight populations

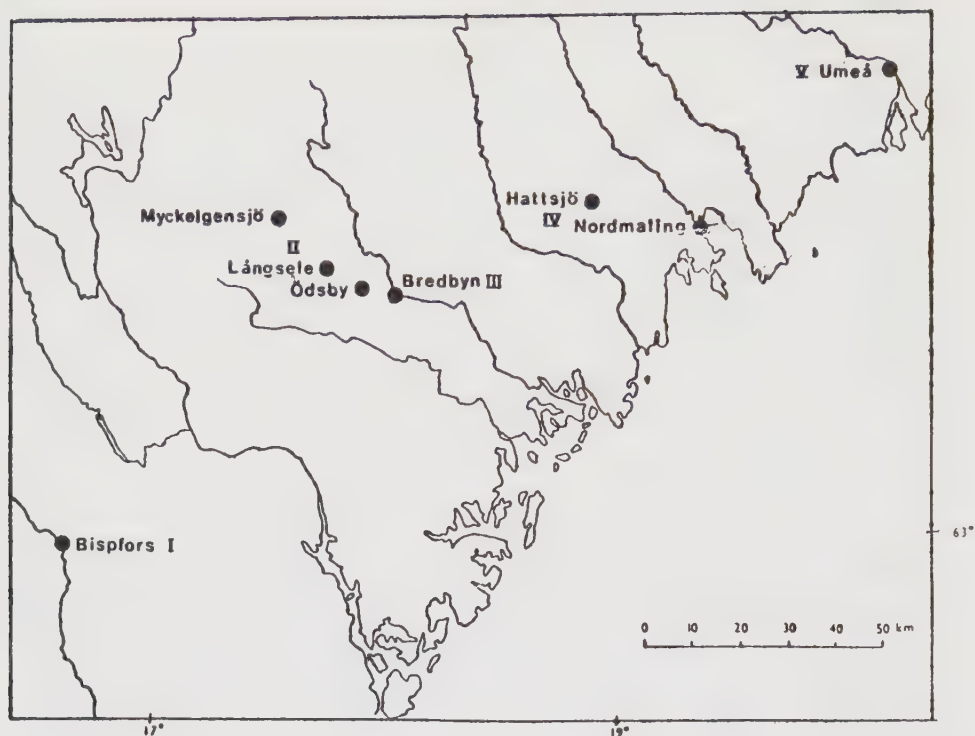


Fig. 6. The investigated geographical region where the central and the northern karyotypic races come in contact. The eight populations have been divided into five areas, which are designated with roman numerals.

have been divided into five areas according to their geographic localization and the karyotypes found in them, in order to make the analysis less complicated (see Fig. 6). In Bispsfors (area I), situated far from the hybrid zone, all chromosomally investigated animals had the M-karyotype. Also in Myckelgensjö, Långsele and Ödsby (area II), near the hybrid zone, only M-karyotypes were found. In Bredbyn (area III) both M-, N- and hybrid animals were found. In Hattsjö and Nordmaling (area IV), only N-race

animals were found. Also in Umeå (area V) only N-race animals were found. Umeå is presumably situated far from the hybrid zone.

The population at Bredbyn contained mostly N-race and hybrid animals. Only one animal had the M-karyotype in this sample (see Table 1). The karyotypic border must, therefore, have its center somewhere between Bredbyn and Ödsby and the width of the karyotypic hybrid zone is here probably less than 10 km.

The number of animals caught in the eight populations and analyzed chromosomally and electrophoretically are given in Table I in paper III. The allele frequencies observed at the three loci Ada, EsB1 and Mpi are listed in Tables 3, 4 and 5 in paper III. No new genetic variation was found in the investigation of the hybrid zone for any of the investigated loci.

Arguments for gene flow

The frequencies found for the three most common alleles at the Mpi locus all describe clinal changes over the contact region when the populations are regarded in linear order from Bispfors (M-race), over the hybrid population at Bredbyn, to Umeå (N-race). The D allele is so rare on both sides of the hybrid zone that it does not give much information in this respect. This parallel change in allele frequencies over the contact region is most easily explained by a flow of genes between the central and the northern karyotypic races.

Another piece of evidence for gene flow over the karyo-

typic border comes from the presence in both karyotypic backgrounds of ten out of the eleven alleles ever detected at the three investigated loci. In the previously described investigation (paper I), the alleles Ada*C, EsB1*B and Mpi*C had not been observed in the northern race. Now, single heterozygotes for these alleles were found in this race within the investigated region. Similarly, the Mpi*D allele had not been observed in the central race in the previous investigation, but was now found here in two animals close to the hybrid zone. Although these alleles are rare and the samples studied in some populations are not very large, their appearance pattern can be taken as an indication of a genetic contact between the two races.

In Fig. 7 (from Fig. 4 in paper III) the observed relative frequencies of the A, B and C alleles at the Mpi locus (the dots with bars) are plotted over the five areas. It is seen that the alleles change in a similar pattern over the areas, with area II (M-race) close to the karyotypic hybrid zone being intermediate in allele frequencies. The explanation that these intermediate frequencies are due to a flow of genes through the karyotypic border, introducing genes from the northern race into the central, could however be wrong if the electrophoretically investigated animals from area II did not all have the M-karyotype. The intermediate allele frequencies would then instead be due to both N- and M-race animals with different allele frequencies existing in this area. To exclude this possibility I have made a table where the allele frequencies at

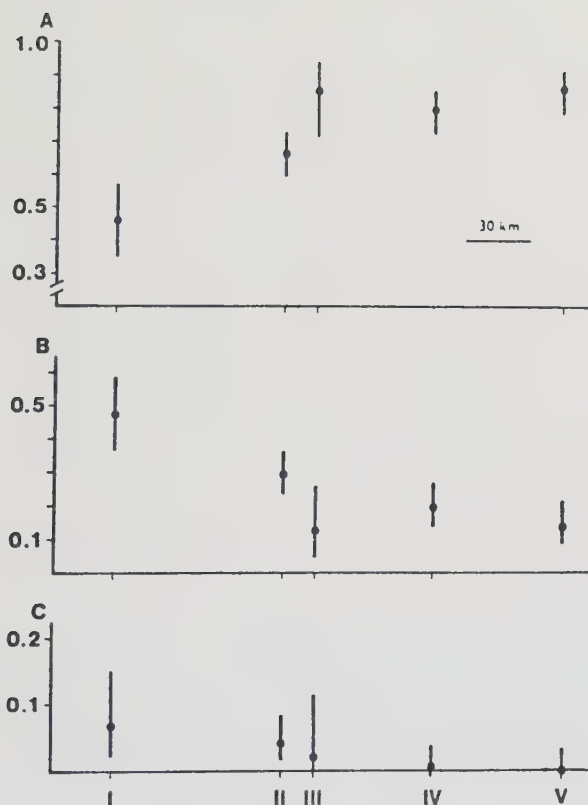


Fig. 7. The change in relative frequencies of the three most common alleles at the Mpi locus over five areas in the contact region between the central and northern karyotypic races in Sorex araneus.

The horizontal axis gives the approximate distances between the areas. The dots show the observed frequencies; note that the scales are different for the C allele and the A and B alleles. The bars through the dots are so constructed that if the true allele frequency lies above (below) the bar, then the probability of getting the observed number of alleles or fewer (more) in the sample is less than 2.5 per cent.

the Mpi locus (the three most informative alleles only) are given, using only karyotyped animals; Table 1 (the karyotypic information is from Fredga pers. comm.). From this table it is clear that the intermediate values observed in the allele frequencies in area II occur also in those animals which for certain are known to have an M-karyotype.

Table 1. Allele frequencies at the Mpi locus for animals with different karyotypes over the five areas in the contact region

Area	NO. of animals karyotyped			Frequency of the <u>A</u> allele			Frequency of the <u>B</u> allele			Frequency of the <u>C</u> allele		
	M	H	N	M	H	N	M	H	N	M	H	N
I	9			0.50			0.50			0		
II	41			0.61			0.33			0.06		
III	1	6	7	a)	0.92	0.79	a)	0	0.21	0	0.08	0
IV			37			0.78			0.20			0.01
V			9			0.78			0.22			0

a) This single animal had an AB genotype

The conclusion to be drawn at this point is that there definitely is some genetic exchange between the central and the northern karyotypic races of Sorex araneus, despite their considerable karyotypic differences.

Restrictions to gene flow

There must, however, be some restrictions to the amount of gene flow over the karyotypic border. This is indicated in particular by the clear difference between the allele frequencies observed between the closely situated areas II (exclusively M-race animals) and III (hybrid population with a majority of N-race animals). If the gene flow had been totally free over the karyotypic border, the frequencies ought to have been more similar in these closely situated areas. Now the gene flow has not been able to equa-

lize the differences at the Mpi locus over such a small distance as 10 km.

Further evidence for a restriction in the amount of gene flow over the karyotypic border comes from the pattern of some of the rare alleles in the contact region. Since they are rare and some of the samples are small, the estimates of their frequencies are uncertain, but a certain pattern can nevertheless be seen. For example, the Ada*D allele was not observed in any of the populations with the N-karyotype (183 animals analyzed), while it was found in 12 animals (185 analyzed) with the M-karyotype and five of these from area II very close to the hybrid zone. And, similarly, the Mpi*C allele was observed in only a single animal on one side of the hybrid zone while it was found in all populations on the other side (see Table 5 in paper I and Table 4 in paper III).

Our knowledge about the gene flow, and also about its limitations, comes primarily from the genetic variation at the Mpi locus. There is a possibility that the restriction in gene flow may be particularly strong for this gene, and does not have a similar effect on the rest of the genome. This would be the case if the Mpi gene lies on a chromosome that is involved in the chromosome barrier. Then it could be difficult for a Mpi-gene to enter the other race, since this would only occur via a crossing-over event which perhaps only seldom happens. Whether the Mpi locus is linked in this way to any of the chromosomes involved in the karyotypic barrier, is not yet known.

OTHER INVESTIGATED KARYOTYPIC HYBRID ZONES IN MAMMALS

A certain amount of gene flow is thus observed between the central and the northern karyotypic races of Sorex araneus, although it is restricted to some degree. Very few studies on the gene flow between chromosomally differentiated populations have been performed, in particular on mammals. As far as I know, only three such species have been studied in the same way as we have studied Sorex araneus, i.e. by finding a genetic marker other than the chromosome differences and using this in an analysis of populations from various distances to the hybrid zone.

Spalax ehrenbergi

There are four karyotypic races of the fossorial mole rat Spalax ehrenbergi in West Asia. Their diploid chromosome numbers are 52, 54, 58 and 60 and these differences are due to Robertsonian rearrangements (Wahrman et al. 1969). Where the races meet, narrow hybrid zones are formed (0.3-2.8 km). Behavioral differences have been observed between the races (Nevo et al. 1975; 1976; Nevo and Heth 1976). Also assortative mating based on smell has been observed in two of the races. However, reproductive isolation can not be very strong since many hybrids are found within the contact zones. Electrophoretic studies (Nevo and Shaw 1972; Nevo and Cleve 1978) show little divergence between the races for 25 analyzed loci, although one alternative fixation

was observed for one locus. In one of the electrophoretic studies, samples were taken from the central parts of the races and from populations near the hybrid zone. It was then found that there probably is a gene flow between the races, since at two informative loci, transferrin and esterase-4, the allele frequencies were intermediate near the hybrid zones compared to the frequencies in the center of the races. This pattern was similar for both the investigated contact zones, one between the 2n=52 and 54 races and the other between the 2n=54 and 58 races (Nevo 1983).

Thomomys bottae

The American fossorial pocket gopher Thomomys bottae is a species with great chromosomal variation between different geographically distributed populations. The karyotypic differences are due to whole arm constitutive heterochromatine additions/deletions. These populations exhibit several hybrid zones, some of which have been investigated both chromosomally and eletrophoretically.

The "White Sacramento contact" between T. b. ruidosae and T. b. actuosis is one of these hybrid zones, investigated by Patton and Yang (1977) and Patton et al. (1979). The different karyotypic subspecies do not overlap and chromosomal hybrids are found in only a narrow belt. When 23 loci were electrophoretically analyzed, three showed nearly alternative allelic fixations between the two forms. The frequency changes of these alleles over the hybrid zone were quite abrupt which indicates at most a limited amount of gene flow between the karyotypically

different subspecies. The authors have, however, been able to show by morphological measurements and by the presence of rare alleles in the "wrong" genetic backgrounds that genetic contact exists over the karyotypic border.

The "Sangre de Cristo contact" between two other chromosomally diverging populations of Thomomys bottae has been investigated electrophoretically and chromosomally by Hafner et al. (1983). The karyotypic difference shows here a clinal pattern in the diploid chromosome number over the 200 km wide hybrid zone, ranging from $2n=76$ to $2n=88$ going from the south to the north. An attempt was made to find an electrophoretic marker separating the two forms, but when 22 loci were analyzed no fixed alleles or any major frequency shifts were found between them. All morphological evidence seems, however, to indicate that gene flow occurs between the karyotypically different populations (Hafner et al. 1983).

Uroderma bilobatum

The tent making bat Uroderma bilobatum, living along the Central American west coast, is a species with two morphologically similar karyotypic races which have been studied chromosomally and electrophoretically by Baker (1981) and Greenbaum (1981). The chromosome differences are here due to three Robertsonian rearrangements involving whole arm fusions and fissions. The zone in which chromosomal variation has been found is very wide, over 400 km. The species has a high vagility and animals can disperse over large distances, which probably accounts for the width of

the zone. The clines in the three chromosomal types are all identical and the major frequency changes occur in a zone of only about 35 km width. When 22 loci were eletrophoretically analyzed, 10 showed some genetic variation and one locus, Es-2, turned out to be clearly polymorphic. The alleles 100 and 94 were about equally common in the $2n=38$ race, while only allele 100 seems to occur in the $2n=44$ race. However, a single animal with this karyotype which was heterozygous for the 94 allele was found in the investigated region. This seems to indicate that genetic exchange can occur between the karyotypic forms. The interpretation of the data from this interesting hybrid zone and the evolutionary processes going on there have been discussed in detail by Baker (1981) and Greenbaum (1981), and also by Barton (1982) and by Hafner (1982).

Summing up the data on the electrophoretic studies of karyotypic hybrid zones in mammals, we can conclude that some gene flow seems to occur across all the investigated karyotypic borders though the information from many of the cases is very scanty. The hybrid zone between the northern and the central karyotypic races in Sorex araneus is, thus, not unique in this respect.

GENETIC DIFFERENTIATION

Despite the considerable chromosomal differences that exist between the karyotypic races of the Swedish shrews, no corresponding genetic differentiation has been found to follow these chromosomal differences. When 27 loci were analyzed electrophoretically (in some cases 35), only one major racial difference was found (the difference between the northern and the other races with respect to allele frequencies at the Mpi locus). This observation tells us that the karyotypic races are genetically very similar, at least in their enzyme producing genes.

To get a measure of the genetic differentiation between the karyotypic races of Sorex araneus, their genetic distances (D) have been calculated. The genetic distance (Nei 1972) is a measure of the mean number of substitutions per locus which have accumulated since two populations separated. The methods used here for estimating distances and their standard errors were given by Mueller and Ayala (1982) and take into account the correction for small samples of animals as given by Nei (1978).

Genetic distances between karyotypic races

Paper II presents the results of a calculation of the genetic distances between pairwise combinations of the three karyotypic races of Sorex araneus in Sweden. The estimates are based on the 27 enzyme loci investigated in paper I.

The D-values and their standard errors are given in Table 5 (paper II). They are all very small and range from 0.004 to 0.019. These low values are not surprising since 26 of the 27 loci analyzed did not show any clear differences between the races. The standard errors are large, depending on both the limited number of investigated loci and the uneven distribution of the variability over loci. Because of the uncertainty in these estimates it is pointless to compare the D-values between the karyotypic races with each other.

Genetic distances between shrew species

The estimated distances for the karyotypic races can, however, be compared to the genetic distances between different species, in order to judge the degree of genetic differentiation between the races.

In paper II the genetic distances between four shrew species, Sorex araneus, Sorex minutus, Sorex caecutiens and Neomys fodiens were estimated. The estimates of the genetic distances and their standard errors were based on the allele frequencies observed at 35 investigated loci (see paper II and the more detailed information given in Table A2a in the Appendix). Table A2a presents the relative mobility of the alleles at the 18 loci for which no genetic variation was found in any of the four shrew species ($p=1.00$). In Table A2b are given the allele frequencies for the remaining 17 loci. In both tables the mobility of the alleles has the most common allele in Sorex araneus as standard. In Neomys fodiens no enzyme activity

was found for the enzymes GPT and CAT. This is here interpreted as being due to fixation of a null allele. As seen in Table A2b, MPI is genetically variable in all the shrew species. This is also the situation in many other animals. Skibinski and Ward (1982) have shown that MPI is one of the enzymes with the highest heterozygosity values, when these are compared over many different species.

The D-values with their standard errors between pairs of the shrew species are given in Table 4 in paper II. The genetic distances range from 0.32 to 0.52 among the Sorex species and from 1.39 to 2.23 between Neomys fo-
diens and the Sorex species. These genetic distances are much greater than the distances observed between the karyotypic races in Sorex araneus (0.004 to 0.019), which can be concluded despite the large standard errors to the estimates. Thus, using the electrophoretically detectable variation as a general measure of genetic differentiation, we can say that the karyotypic races of Sorex araneus are much more genetically similar to each other than are any of the different investigated shrew species.

Genetic differentiation between hybridizing taxa

It is interesting to see if the close similarity between the karyotypic races in Sorex araneus makes this species unique when compared to other species with electrophoretically investigated karyotypically different populations. In Table 2 is given a brief summary of the available data

on electrophoretically investigated small mammals showing karyotypic variation between populations. Only such examples have been included where different karyotypic forms within a species come in parapatric contact with each other. The table is a reprint of Table 6 in paper I with the addition of two recent studies (Hafner et al. 1983; Baverstock et al. 1983). From the table it can be seen that the genetic differentiation detected between the karyotypic races of Sorex araneus is of the same magnitude as that found in many other mammalian species in the same situation. Of the eight other parapatric situations surveyed in the table, six show only little genetic differentiation between the karyotypic forms. These are: Mus musculus, Proechimys guaire, Spalax ehrenbergi, Thomomys bottae (Sangre de Cristo), Rattus rattus and Uroderma bilobatum. Five of these differ chromosomally by Robertsonian rearrangements as do the shrew races. There are, thus, many examples of karyotypically different populations which show very little genetical divergence when judged by electrophoretic criteria.

One should, however, remember the possibility that the karyotypic races may be more genetically differentiated than the enzyme studies tell us. Even if the enzyme loci analyzed are randomly chosen, they only represent a limited sample of the structural genes in the genome. There may be differences in other genes which are not translated into enzyme proteins. For example, in the grasshopper Podisma pedestris Barton and Hewitt (1981 b) have shown that the hybrid inviability observed between two karyo-

Table 2. Survey of electrophoretic data on chromosomally different races (subspecies) in mammalian species. The information is, whenever possible, quoted directly from the cited articles. Only such taxa are included in the table that are presumed to be in parapatric contact

Order	Species	Type of chromosomal differences	No. of karyotypic races studied	Geographic distribution	Morphology
Rodentia	<u>Mus musculus</u> (alpine system)	Robertsonian changes	3	Parapatric distribution with contact zones	- c)
	<u>Proechimys guaire</u>	One Robertsonian translocation and three pericentric inversions	4	Parapatric distribution with abrupt transitions	similar
	<u>Spalax ehrenbergi</u>	Robertsonian changes and pericentric inversions	4	Clinally and parapatrically distributed populations with three narrow contact zones	similar
	<u>Thomomys bottae</u> (White-Sacramento)	Whole arm constitutive heterochromatin additions and deletions	2	Parapatric distribution with a narrow contact zone	clear differences in size and colour
	<u>Thomomys bottae</u> (Sangre de Cristo)	-"-	2	Parapatric distribution with a broad contact zone	differences in colour & morphology
	<u>Thomomys talpoides</u>	Robertsonian changes and/or pericentric inversions	6	Parapatric distribution with several contact zones	total similarity
	<u>Rattus rattus</u>	One centric fusion/fission	2	Parapatric distribution with one contact zone	- c)
Chiroptera	<u>Uroderma bilobatum</u>	Three chromosome translocations involving whole arm fusions or fissions between telomeric and/or centromeric regions	2	Parapatric distribution with a broad hybrid zone	indistinguishable
Insectivora	<u>Sorex araneus</u>	Robertsonian translocations	3	Parapatric distribution with two contact zones	no obvious differences

a) The criterion for polymorphic loci may differ between authors

b) This column does not include the cases given in the previous column

No. of individuals studied	No. of loci studied	No. of loci polymorphic in at least one race ^{a)}	No. of loci with substitutional differences between populations	No. of loci where the most common allele differs among races ^{b)}	References
75	29	7	0	0	BRITTON-DAVIDIAN et al. (1980)
83	22	11	0	2	BENALDO et al. (1979) REIG et al. (1979)
383	17	7	0	2	WAHREN et al. (1969) NEVO and SHAW (1972)
499	8 ^{d)}	2	1	0	NEVO and CLEVE (1978)
92	23	5	3	2	PATTON et al. (1979) PATTON and YANG (1977)
109	22	10	0	0	HAFNER et al. (1983)
276	31	23	2	5	NEVO et al. (1974) THAELER (1974)
6	45	6	2	1	BAVERSTOCK et al. (1983)
366	22	11	0	0	BAKER (1981) GREENBAUM (1981)
225	27	3	0	1	FRYKMAN et al. (1983)

c) Not investigated by the authors, but the animals are known to be similar

d) These loci are different from the ones studied earlier

typic races is due to a large number of gene differences. However, in an electrophoretic analysis of the same races on 20 enzyme loci, no genetic difference between the races was found.

In S. araneus we have information about the similarity of the karyotypic races not only from the electrophoretic study. We also know that the races are similar in genes determining morphology, since it is not possible to distinguish the races from each other by any morphological characteristic. The races are probably also similar in many of the genes that are involved in the behavioral pattern. Animals of the N-race seem to mate relatively freely with animals of the M-race, since hybrids are formed in nature. The races do not overlap over large distances and the hybrid zone is obviously narrow. This is not only due to the low vagility of the species. Barton and Hewitt (1981 a) have pointed out that if animals in a hybrid zone prefer to mate with other animals of the same type, then the zone will be broad. If, on the other hand, animals mate at random, the hybrid zone will be narrow.

Thus, all the available evidence indicates that the central and northern karyotypic races in Sorex araneus are genetically very similar.

CONCLUSIONS

We have now seen that there is gene flow between the central and the northern karyotypic races of Sorex araneus. We have also seen that despite the extensive karyotypic differences between them they are not very genetically differentiated. The questions why the races are so similar must then be due to one of only two causes (see paper I):

1. The populations did never diverge during the time they were separated and acquired the chromosomal differences.
2. Evolutionary divergence occurred during the allopatric period, but has subsequently been equalized by gene flow during the time of the present contact.

Now I will propose that the second alternative can be excluded. The effect of the gene flow has not yet even been able to equalize the differences in the allele frequencies at the Mpi locus over the short distances within the investigated contact region. A rough calculation (Bengtsson, pers. comm.) based on Barton (1979 a), shows that if the mean vagility of the animals is 800 m and the contact between the races has existed for 10 000 years, then selectively neutral genetic differences between the races will have become mixed within a region of less than 100 km from the hybrid zone. This implies, for example, that the gene flow will hardly have affected the populations at Umeå and Bispfors, with respect

to any selectively neutral genetic variation. The same argument holds even more for comparisons of the populations at Ammarnäs (N) and Mora or Uppsala (M-race).

The conclusions we can draw is thus, that not only are the two karyotypic races at present in genetic contact through gene flow over the karyotypic border; they must also have been genetically similar when they came in contact with each other about 10 000 years ago.

Will the karyotypic races become different species?

A chromosomal barrier of the type that is observed in Sorex araneus is not easy to get rid of, once it is established (Barton 1979 b). What can happen to such a stable hybrid zone, once it has become formed? (For general discussions of hybrid zones see Bigelow 1965; Key 1968, 1974; Barton and Hewitt 1981 a, 1983.)

1. The hybrid zone can move until one of the races disappears and with that the hybrid zone. If one race has a selective advantage over the other, the zone will move forward in favour of the superior type. Or if there are more individuals on one side of the zone, it will also move, then towards regions of low population density (Barton and Hewitt 1981 a). Such a movement of the hybrid zone between the central and the northern karyotypic races of the Swedish shrews is a possible evolutionary pathway. The process will of course take a lot of time but can result in the disappearance of one of the races, perhaps most likely the central one.

2. The hybrid zone can be dissolved. If, for example, all the chromosomes that are involved in the karyotypic barrier become telocentric, then this will be the case. Since selection probably favours animals with many telocentrics in the contact zone due to the fertile hybrids they can produce, also this is an imaginable future for the central and the northern karyotypic races in Sweden. But again, also this process will take a long time to go to completion.

3. The hybrid zone can be strengthened, and in course of time reach the level of a species barrier. For example, selection may act on the races to evolve a preferential mating behaviour which leads to complete assortative mating, and thereby make it possible for the races to invade each others ranges as different species. For the karyotypic races of the common shrew in Sweden this will be very difficult. First of all the karyotypic races were so genetically similar when they came into contact that there is probably little genetic variation between them which can be used by selection to strengthen the existing barrier. And secondly, the gene flow between the races will always act against attempts by selection to build new genetic differences between the races.

In the light of what we now know about the karyotypic races of Sorex araneus in Sweden, I would therefore like to conclude: If they are on their way to become separate species, they have a very long way to go.

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In this investigation of shrews I have worked together with three colleagues and friends. Bengt Olle Bengtsson initiated the electrophoretic study of shrews and has acted as my supervisor after I got involved in the project. With his lucidity and ability to put ideas in the right perspective he has helped me both to start and finish this thesis. Karl Fredga has always gladly shared his knowledge about shrews, and has with never-failing enthusiasm set traps, made and analyzed chromosome preparations, and closely scrutinized my manuscripts. Vibeke Simonsen opened her laboratory in Århus for me, and has guided me through the electrophoretic morass with firm encouragement. I am most deeply indebted to them all.

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APPENDIX

Table A1. List of electrophoretically analyzed animals

Locality	Year	<u>N.f.</u>	<u>S.c.</u>	<u>S.m.</u>	<u>S.a.</u>
Stensoffa	1979			10	14
	1980				19
	1981				13
Stensjön	1980				24
	1982				5
Uppsala	1979				5 a)
	1980				42 a)
Mora	1980				18
Nordingrå	1982				5
Bispfors	1977				11 b)
	1982				26
Myckelgensjö	1977				5 b)
	1981				23
	1982				1
Långsele	1982	1		1	19
Ödsby	1982	6		4	50
Bredbyn	1981				16
	1982	2			8
Hattsjö	1981				34
Nordmaling	1981				40
Umeå	1979				14 c)
	1980				19
	1981				25
Amnarnäs	1980				53
	1981		7		4

a) Trapped by Fredrik Karlsson

b) Trapped by Karl Fredga

c) Trapped by Jan Nygren

Table A2. Genetic variation at 35 enzyme loci in four shrew species. The most common allele in S.a. has been used as standard. The dash designates that no enzyme activity was obtained for any of the animals of the given species

A2a. Enzyme loci for which no genetic variation was found in any of the species

Enzyme	Locus	<u>S. araneus</u> (N=10-15)	<u>S. minutus</u> (N=5-15)	<u>S. caecutiens</u> (N=7)	<u>Neomys fodiens</u> (N=9)
Aconitase	<u>Aco2</u>	-100	-100	-100	-140
Adenylate kinase	<u>Ak1</u>	-100	-100	-100	-120
	<u>Ak2</u>	100	62	100	85
Esterase	<u>EsB3</u>	100	100	100	80
Fumarate hydratase	<u>Fh</u>	100	100	100	80
Glutamic-oxaloacetic transaminase	<u>Got2</u>	-100	-100	-100	-100
Glucose-6-phosphate dehydrogenase	<u>Gpd</u>	100	100	120	200
Glucose phosphate isomerase	<u>Gpi</u>	-100	-100	-25	-33
Glutamic-pyruvic transaminase	<u>Gpt</u>	100	60	40	-
Lactate dehydrogenase	<u>LdhB</u>	-100	-100	-100	-67
Malate dehydrogenase	<u>Mdh1</u>	100	100	100	71
	<u>Mdh2</u>	-100	-100	-100	-89
Malic enzyme	<u>Me1</u>	100	100	100	70
	<u>Me2</u>	-100	-100	-100	100
Purine-nucleoside phosphorylase	<u>Np</u>	100	100	100	83
Peptidase	<u>PepA</u>	100	92	100	69
Phosphoglycerate kinase	<u>Pgk</u>	100	100	100	100
Superoxide dismutase	<u>Sod</u>	-100	-100	-100	-100

A2b. Allele frequencies at the enzyme loci where genetic variation was found in at least one of the species

Enzyme	Locus	Allele	Allele frequencies			
			<u>S. araneus</u> (N=10-100)	<u>S. minutus</u> (N=5-15)	<u>S. caecutiens</u> (N=7)	<u>N. fodiens</u> (N=9)
Aconitase	<u>Aco1</u>	37				1.00
		100	0.95	1.00		
		150	0.05			
		175			1.00	
Adenosine deaminase	<u>Ada</u>	10				1.00
		80		0.87		
		100	1.00			
		120		0.13		
		180			1.00	
Alcohol dehydrogenase	<u>Adh</u>	-100	1.00			
		-83		0.17		
		-67		0.83		
		-50			1.00	1.00
Catalase	<u>Cat</u>	-33		0.10		-
		-100	1.00	0.90	1.00	-
Esterase	<u>Esa1</u>	94	0.02		1.00	1.00
		100	0.98	1.00		
	<u>Che</u>	89	0.06			
		94			1.00	1.00
		100	0.94	1.00		
	<u>EsB1</u>	null	0.11	0.97		
		74			0.86	
		78			0.14	
		93		0.03		1.00
		100	0.89			
	<u>EsD</u>	60		0.97	1.00	1.00
		100	1.00			
Glutamic-oxaloacetic transaminase	<u>Got1</u>	63				1.00
		100	0.99	1.00	1.00	
		122	0.01			
Isocitrate dehydrogenase	<u>Idh1</u>	66	0.01			
		100	0.98	1.00	1.00	
		133	0.01			1.00
	<u>Idh2</u>	100	1.00	0.93	1.00	
		150		0.07		
		250				1.00
Lactate dehydrogenase	<u>Ldh A</u>	100	1.00	0.97	1.00	
		114		0.03		1.00
Mannose phosphate isomerase	<u>Mpi</u>	60	0.02			0.67
		80				
		100	0.80	0.97	0.29	0.33
		130				
		150	0.18	0.03		
Peptidase	<u>PepC</u>	190			0.71	
		85				1.00
		90	0.03	1.00		
		100	0.97			
		108			1.00	
6-phosphogluconate dehydrogenase	<u>Pgd</u>	67	0.01			
		78		1.00	1.00	
		88				1.00
		100	0.97			
		133	0.02			
Phosphoglucosmutase	<u>Pgm</u>	65			0.07	0.56
		75	0.01		0.93	
		85				0.44
		100	0.99			
		110		0.07		
		125		0.93		
		133	0.01	1.00		
Nonspecific dehydrogenase	<u>Ndh</u>	100	0.99			
		133	0.01			
		166			1.00	1.00

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